Application No.: 10/572,989 Filed: 01/25/2007

Applicants: Robert J. Collier et al.

Examiner: Joanne Hama

Office Action Dated: October 7, 2008 Response Dated: October 28, 2008

## Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

## **Listing of Claims:**

- 1. (Currently Amended) A method for breeding cows for desired milking characteristics comprising the steps of:
- a<sub>-</sub>) identifying the allele of the bovine beta2-adrenergic receptor (ADRB2) gene in at least one potential parent <u>bull animals</u> <u>by isolating DNA from the bull and</u> screening the DNA to identify a variant allele of ADRB2 with an adenine (A) nucleotide at <u>position 11 inclusive of the start codon ATG (an A11C allele)</u>; and
- b-) breeding the bull identified as having the A11C allele male and with a female cow animals to create a daughter cow having at least one allele of beta2 adregenic receptor the ADRB2 gene associated with improved milking characteristics.
- 2. (Currently Amended) The method of claim 1 wherein the method for identifying the allele includes isolating DNA from the parent and screening with a method is selected from the group consisting of direct sequencing, primer extension, restriction length fragment polymorphism, and allele-specific hybridization.
- 3. (Currently Amended) The method of claim 1 whereby the screening method is intending to identify A11C alleles wherein the allele is identified by combining the DNA with a restriction enzyme specific for CCCGGG for a sufficient time to produce a mixture of DNA fragments, applying the DNA fragment mixture to a gel and permitting migration of the mixture components for a time sufficient for them to separate and observing the sizes of DNA on the gel, with the largest fragments being correlated with the A genotype and with better somatic cell score (SCS) phenotype, which corresponds to faster milking speed, and the smaller fragments being associated with the C genotype and less desirable SCS phenotype.

Application No.: 10/572,989 Filed: 01/25/2007

Applicants: Robert J. Collier et al.

Examiner: Joanne Hama

Office Action Dated: October 7, 2008 Response Dated: October 28, 2008

4. (Currently Amended) A method of identifying a bull whose daughter cows will have a faster milking time, the method comprising the steps of

- a-) obtaining a sample of DNA from a bull;
- b-) combining the DNA with a pair of PCR probes comprising the SEQ ID NOs: 1 and 2 or SEQ ID NOs: 3 and 4, wherein the probes flank the 11th nucleotide position of a bovine beta2-adrenoreceptor gene coding sequence inclusive of the start codon ATG, the probes enable detection of a single nucleotide polymorphism at said 11th nucleotide position;
- c<sub>-</sub>) incubating the DNA under conditions permitting the DNA bounded by the PCR probes to produce DNA amplicons;
  - d-) isolating the DNA amplicons;
- e.) combining the DNA amplicons with a restriction enzyme specific for to CCCGGG for a sufficient time to produce a mixture of DNA fragments from the amplicons comprising CCCGGG;
- f<sub>-</sub>) applying the DNA fragment mixture to a gel and permitting migration of the mixture components for a time sufficient for them to separate; and
- g<sub>-</sub>) observing the sizes of DNA on the gel, with the largest fragments being correlated with the A genotype and with better somatic cell score (SCS) phenotype, and the smaller fragments being associated with the C genotype and less desirable SCS phenotype, wherein the bull whose daughter cows will have a faster milking time which corresponds to a faster milking speed.
- 5. (Currently Amended) A milking attribute PCR-RFLP kit, which comprises: a pair of primers, which flank the 11th nucleotide position of the bovine beta2-adrenoreceptor gene coding sequence inclusive of the start codon ATG, the primers enable detection of a single nucleotide polymorphism at said 11th nucleotide position; and a restriction enzyme specific for the CCCGGG site.
  - 6. (Original) The kit of claim 5 wherein the restriction enzyme is SmaI.

Application No.: 10/572,989 Filed: 01/25/2007 Applicants: Robert J. Collier et al. Examiner: Joanne Hama

Office Action Dated: October 7, 2008 Response Dated: October 28, 2008

(Currently Amended) The kit of claim 5 wherein the primer pairs are selected 7. from pair 1 (SEQ ID NOs: 1 and 2) or from pair 2 (SEQ ID NOs: 3 and 4).